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Epidemiology of canine distemper virus in wild raccoon dogs (*Nyctereutes procyonoides*) from South Korea

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ABSTRACT

Raccoon dogs (*Nyctereutes procyonoides*) are widespread and common in South Korea. In 2011, we obtained serum samples from 102 wild raccoon dogs to survey their exposure to canine distemper virus (CDV). Forty-five of the 102 animals (44.1%) were seropositive. Field cases of canine distemper in wild raccoon dogs from 2010 to 2011 were investigated. Fourteen cases of CDV infection were identified by a commercially available CDV antigen detection kit. These cases were used for virus isolation and molecular analysis. Sequence analysis of hemagglutinin genes indicated that all viruses isolated belonged to the Asia-2 genotype. H protein residues which are related to the receptor and host specificity (residues 530 and 549) were analyzed. A glutamic acid (E) residue is present at 530 in all isolates. At 549, a histidine (H) residue was found in five isolates and tyrosine (Y) residue was found in 6 isolates. Our study demonstrated that CDV infection was widespread in wild raccoon dogs in South Korea.

1. Introduction

Canine distemper is a disease that is distributed worldwide and highly contagious and fatal in dogs and other species of the Canidae, Mustelidae, Procyonidae, Ursidae and the large Felidae families [1]. Symptoms can be acute or subacute and manifested as a generalized infection, respiratory disease, hyperkeratosis, central nervous system disturbance, or a combination of these [2]. The etiological agent, canine distemper virus (CDV), is a negative sense, single-stranded RNA virus belonging to the genus *Morbillivirus*, family *Paramyxoviridae*. The CDV genome encodes seven proteins. Of these, the fusion (F) and hemagglutinin (H) envelope proteins are involved in viral induced cell-to-cell fusion, cytopathogenicity, and cell tropism [3–6]. The spread of CDV to novel host species may be associated with adaptation at signaling lymphocytic activation molecule (SLAM) receptor-binding sites 530 and 549 of the H gene [7,8]. Substitutions in these two sites are predominantly related to host switches to non-canine hosts. The substitutions involve glycine (G) or glutamic acid (E) at site 530 to aspartic acid (D) in canine samples, asparagine (N) or arginine (R) in non-canine hosts, and substitutions of tyrosine (Y) at site 549 in canine samples to histidine (H) in other hosts [8,9]. Recently, the incidence of CDV in the wildlife has been on the rise, presenting a serious problem [10–12].

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Raccoon dogs (*Nyctereutes procyonoides*) are common and widely distributed in the Far East (Amur-Ussuri region in Russia as well as parts of China, northern Vietnam, Korea, and Japan) [13]. It is the only extant species in the genus *Nyctereutes*. It is considered a basal canid species, resembling ancestral forms of the family. Among the Canidae, the raccoon dog regularly climbs trees, a characteristic shared with the North American gray fox, another basal species. In South Korea, the raccoon dog is one of the most abundant mammals, and 35% of casualty cases treated in wildlife rescue centers are due to this species, which is the highest among mammals [14,15]. This species represents the second highest incidence of road-kill [16], which is alarming from an epidemiologic perspective, since due to the increased potential for transmission of disease from carnivores (companion animals) to raccoon dogs, if the animals are in contact with infected carnivores. Moreover, given their tendency to wander [17], they have increased contact with domestic animals, wild animals, agricultural operations, and humans [18]. Until recently, there were no specific sero-surveillance programs for CDV in wild raccoon dogs in South Korea and only one report has addressed CDV in these animals. Little is known of the prevalence of CDV in wild raccoon dogs and their risk of CDV transmission.

Thus, this study was conducted to investigate the distribution and degree of risk for CDV in wild raccoon dogs by surveying the serologic prevalence of CDV in 2011. In addition, to obtain information about the strains of CDV in South Korea, the phylogenetic relationship of CDV strains recovered from 11 wild raccoon dogs were investigated using the CDV H and F gene sequences. We compared the H gene sequences from the obtained CDV isolates with the published gene sequence from terrestrial carnivore species worldwide, focusing on residues at 530 and 549 in CDV strains.

2. Materials and methods

2.1. Serum samples

One hundred two serum samples were collected from wild raccoon dogs captured around the Chonbuk province (60 samples) and the surrounding areas (42 samples) in 2011. All samples were inactivated by incubation at 56 °C for 30 min and then kept at −20 °C until used.

2.2. Virus neutralization (VN) test

CDV (Onderstepoort) was propagated in monolayer cultures of Vero cells (African green monkey kidney cells). Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 7% fetal calf serum (FCS) and antibiotics at 37 °C in an atmosphere of 5% CO_2. For the screening of CDV antibodies, 2-fold dilutions of serum were added to the working virus dilution containing 100 units of 50% tissue culture infectious dose ($TCID_{50}$) of CDV and incubated at 37 °C for 1 h. Thereafter, each mixture was added to duplicate microtiter wells of subconfluented monolayers of Vero cells. Each plate was incubated at 37 °C for 1 h and washed twice with FCS-free DMEM, prior to the addition of DMEM containing 7% FCS. The plates were incubated for up to 4 days. Titers ≥ 1:8 were considered positive [19].

2.3. Samples and history

Sixty-two wild raccoon dogs were rescued from the Chonbuk and Chungnam provinces in 2010 and 2011. Some presented with clinical signs consistent with CDV infection, including prostration, immobility, coughing and anorexia. To detect the canine distemper virus antigen in conjunctiva, urine, serum or plasma, we used a commercial CDV antigen detection kit (BioNote, Inc., Korea), according to the manufacturer's instructions. Nasal discharge, rectal swab, lung and spleen tissues from the affected raccoon dogs were acquired for virus isolation.

2.4. Virus isolation

DMEM containing high concentrations of antibiotics was added to swabs and tissues and mixed by vortexing. The samples were centrifuged and the supernatants were filtered through a 0.45 μm filter. The filtrates were used to inoculate Vero cells. The inoculated cells were cultured until a cytopathic effect was observed. The virus harvest fluid was kept at −80 °C until used.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR) and CDV detection

Total RNA was extracted from the virus fluid using Easy-BLUE™ total RNA extraction kit (Intron Biotechnology, Korea) according to the manufacturer's instructions. The extracted RNA was eluted using 30 μl of diethyl pyrocarbonate-treated water. RT was carried out with a random 9-mer primer using an RNA LA PCR kit (AMV) (TaKaRa Bio, Japan). For routine CDV detection, a set of primers specific for the H gene interior was applied: CDV H13 (5′-CAAGACAAGGTGGGTGCCTT-3′) and CDV H18 (5′-CTTGGTGAAATCGAACTCCA-3′) [20]. Amplification was performed using pre-denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 30 s, followed by a final extension step of 5 min at 72 °C.

2.6. H gene sequencing

The complete H gene was amplified using primers HP-1F (5′-AGGCAGGCAATACCCTGATA-3′) and HP-4R (5′-TAATAATGCTCCCGATTTCGA-3′). Amplification was performed with a pre-denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 3 min, followed by a final extension step of 7 min at 72 °C. The amplified products were cloned into the pGEM-T Easy Vector (Promega, USA) and nucleotide sequences were determined using an ABI PRISM 310 Genetic Analyzer autosequencer (Applied Biosystems, USA). For sequence analysis, the H gene primers HP-2F (5′-GAATTTAGCAGA TTGCTGAAAGAG-3′), HP-2R (5′-CAGTGCTCTCTTCTACAC ACAAGG-3′) and HP-3R (5′-GGGGTATCACAGTGAACTGGT C-3′) were used.